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Original paper

Detection of *Fusarium* damaged kernels in Canada Western Red Spring wheat using visible/near-infrared hyperspectral imaging and principal component analysis

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ABSTRACT

Fusarium damage in wheat reduces the quality and safety of food and feed products. In this study, the use of hyperspectral imaging was investigated to detect fusarium damaged kernels (FDK) in Canadian wheat samples. Eight hundred kernels of Canada Western Red Spring wheat were segregated into three classes of kernels: sound, mildly damaged and severely damaged. Singulated kernels were scanned with a hyperspectral imaging system in the visible-NIR (400–1000 nm) wavelength range. Principal component analysis (PCA) was performed on the images and the distribution of PCA scores within individual kernels measured to develop linear discriminant analysis (LDA) models for predicting the extent of fusarium damage. An LDA model classified the wheat kernels into sound and FDK categories with an overall accuracy of 92% or better. Classification based on six selected wavelengths was comparable to that based on the full-spectrum data.

1. Introduction

Fusarium head blight (FHB), also known as scab or tombstone, is a disease of small grain cereals such as wheat, barley, and oat (Gallenberg, 2002). The principal causal agent of FHB is *Fusarium graminearum* Schwabe (Goswami and Kistler, 2004). FHB is favoured by wet weather at flowering, but can infect the grain until harvest, given suitable conditions for infection. However, infection at the early stages of seed development causes the greatest physical damage to the seed and the highest levels of mycotoxin production (McMullen et al., 1997; Windels, 2000). In Canada, kernels infected with *Fusarium* spp. are called fusarium damaged kernels (FDK). As the result of *Fusarium* spp. infection, the kernels may also contain mycotoxins such as deoxynivalenol (DON), also known as vomitoxin (http://www.gipsa.usda.gov/GIPSA/documents/GIPSA_Documents/b-vomitox.pdf). A positive correlation between scab damage and DON levels has been found (Teich et al., 1987). Fusarium infection may have detrimental effect on flour color, ash content, and baking performance (Dexter et al., 1996) as well as other quality and safety issues (Dexter and Nowicki, 2003). In the Canadian grading system, fusarium-damaged wheat is typically characterized by thin or shrunken chalk-like kernels. Fusarium-damaged kernels have a white or pinkish mould or fibrous growth (http://www.grainscanada.gc.ca/oggg-gocg/04/oggg-gocg-4e-eng.htm#r), contrasting with the USDA definition which considers only those kernels that are chalk-like as scabby or tombstone kernels. In the current Canadian grading system, a representative sample is visually inspected for kernels showing evidence of *Fusarium* spp. infection. This process is slow when only slight damage is apparent as inspectors have to use a 10× magnifying lens to examine each suspect seed for the degree of mould growth. In contrast, severely fusarium damaged wheat is typically characterized by thin or shrunken chalk-like kernels easily detected by human inspectors. Visual detection of fusarium damage at an early stage is challanging. Fast and accurate instrumental methods are required to meet the needs of grain industry.

Several laboratory methods are available for measuring DON concentrations in wheat meal and flour including thin-layer chromatography (Fernadez et al., 1994), liquid chromatography (Chang et al., 1984), gas chromatography (Tacke and Casper, 1996), mass spectrometry (Scott et al., 1981) and enzyme-linked immunosorbent assay (Casale et al., 1988). However, these methods are not suitable for rapid online inspection and quality control protocols. Rapid inspection or sorting methods for grain are typically based on kernel density using a gravity table (Tkachuk et al., 1991) or optical properties (Ruan et al., 1998). In a recent preliminary research, Takenaka et al. (2009) have reported encouraging results on DON decontamination using a combination of gravity separation and optical sorting. Williams (1997) used near-infrared (NIR) spectroscopy to model DON levels in whole grain bulk samples with only moderate success. Dowell et al. (1999) investigated

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NIR reflectance to measure DON concentration in single kernels ($r^2 = 0.64$, SE = 44 ppm). Using NIR spectroscopy of individual kernels, sound and FDK were segregated with high accuracy (95–97%) under controlled laboratory conditions (Delwiche and Hareland, 2004). In contrast, test results using this technique on commercial samples under commercial sorting operational conditions had a much lower accuracy (50%; Delwiche, 2008). Peiris et al. (2009) examined NIR absorbance characteristics of various concentrations of DON as well as of sound and fusarium damaged single wheat kernels. This study indicated that NIR spectrometry in the 1000–2100 nm range could estimate DON levels in kernels having more than 60 ppm DON. However, further investigations were required to detect DON in asymptomatic kernels.

Hyperspectral imaging in the shortwave infrared range (1000–2500 nm) has shown potential for detecting fungal contamination in wheat (Zhang et al., 2007; Singh et al., 2007) and fusarium damage in maize corn (Williams et al., 2010) and wheat (Polder et al., 2005). The extremely high cost of cameras sensitive in the shortwave infrared range has been a limiting factor in the development of commercially viable application systems in this waveband range. Berman et al. (2007) have shown that accuracy of fungal detection in wheat grain with hyperspectral imaging over the 420–1000 nm wavelength range could be just as good as over the full spectral range of 420–2500 nm. Recently, the use of high-power bichromatic light emitting diodes (LEDs) emitting at red and green wavelengths have been reported to achieve moderate levels of overall accuracies (50–85%) for detection of fusarium damaged wheat kernels (Delwiche, 2008; Yang et al., 2009).

The objectives of this study were: (a) to investigate the use of hyperspectral imaging in the visible-NIR spectral range (400–1000 nm) for the detection of varying degrees of fusarium damage in wheat, and (b) to identify a reduced set of wavelengths/wavebands to be used in a future development of a low cost imaging system.

2. Materials and methods

2.1. Samples

A set of four hundred individual kernels of Canada Western Red Spring (CWRS) wheat were hand picked from commercial samples containing a range of FDK types. These kernels were individually inspected and scored by a trained grain inspector (Industry Services Division, Canadian Grain Commission) as sound (SND) or FDK. The FDK kernels were further classified into severely damaged (SVR) and mildly damaged (MLD) categories based on the extent of fusarium damage. Mild damage was characterized as chalky-white kernels with fungal or mycelial growth around the germ and in the broadened crease, while severe damage was characterized as shrivelled chalky-white kernels with abundant mycelial growth on both seed surfaces with some pink discoloration at the germ. Kernels with no visible symptoms of damage were characterized as sound. This set of kernels was termed as the calibration set consisting of 200 SND and 200 FDK (100 MLD, 100 SVR) kernels. Another independent set of four hundred kernels, the validation set, was collected from the 2009 harvest survey samples of CWRS wheat received at the Grain Research Laboratory (GRL, CGC). The validation set consisted of 200 SND and 200 FDK (100 MLD, 100 SVR) kernels.

2.2. Hyperspectral imaging system

A push-broom type hyperspectral imaging system (VNIR 100E; Lextel Intelligence Systems, Jackson, MS, USA) in the visible-NIR wavelength range (400–1000 nm) was used for spectral measurements of wheat kernels. The imaging system consisted of a prism-grating-prism spectrograph (ImSpector V10E; Specim, Oulu, Finland), a high-resolution 14-bit CCD camera (PCO Imaging, Germany), a motorized C-mount focusing lens, and a personal computer. The motorized lens assembly moved in front of the camera allowing for imaging stationary samples. Two 250 W quartz-tungsten-halogen lamps were used for sample illumination. Power to each lamp was regulated through a radiometric power supply (M-69931; Newport Oriel, Stratford, CT, USA).

2.3. Image acquisition and calibration

For imaging, singulated wheat kernels, in batches of 24–36 per image, were placed crease-down on a neutral-grey plastic board and hyperspectral images (also known as hypercubes) were collected in the diffuse reflectance mode. Each hypercube contained three rows of kernels: sound (bottom row), mildly damaged (middle row), and severely damaged (top row). Image size was 800 by 400 pixels by 218 wavebands within 400–1000 nm range at a spectral resolution of approximately 2.75 nm and a spatial resolution of 0.028 mm per pixel in both x and y directions. The exposure time was set at 60 ms. Each kernel was approximately 1800 pixels in area.

Dark current and white light reference images were collected before imaging each sample to calibrate spectra at each pixel as percent reflectance value. A polytetrefluoroethylene panel with 99% reflectance (Spectralon, Labsphere, USA) was used to collect white light reference images. Dark current response images were collected with the lamp off and a cap covering the focusing lens. Calibrated reflectance images (R) were calculated using Eq. (1).

$$R = \frac{I_{\text{raw}} - I_{\text{dark}}}{I_{\text{white}} - I_{\text{dark}}} \quad (1)$$

where, I_{raw} is the non-calibrated original image of a sample, I_{white} is the image of the white reference, and I_{dark} is the dark current image. Calibrated hypercubes were subset to keep 181 bands between 450 and 950 nm for further analyses. Data below 450 nm or above 950 nm were excluded due to the presence of excessive noise in this range.

2.4. Spectral characteristics and kernel-background separation

In order to separate kernel objects from the image background, a threshold value was determined based on spectral differences between kernels and image background. Representative spectra of wheat kernels and image background were extracted and visually observed using the region of interest (ROI) tool in the ENVI software (Version 4.5; ITT Visual Information Solutions, Denver, CO, USA). Three sound kernels were manually selected at random from an image to represent 'SND' category (Fig. 1a). Similarly, three mildly damaged kernels and three severely damaged kernels were selected to represent 'MLD' and 'SVR' categories, respectively. For the image background (BKG), roughly 2000 pixels in groups of 400–500 pixels at different locations in an image were selected to represent 'BKG' category. For each category, a representative spectrum was computed as the average of all pixel spectra in the respective category (Fig. 1b). Based on the spectral differences between wheat kernels and the image background as shown by the representative spectra, a threshold value was determined to separate kernel objects from the image background to exclude image background from subsequent analyses. A binary mask image was created for each hypercube by thresholding the image band at 600 nm where pixel intensities greater than 10 were labelled as kernels (white) and values less than 10 were labelled as background (dark) (Fig. 1c). The kernel spectra were mean-normalized by dividing each spectrum with its mean value computed along